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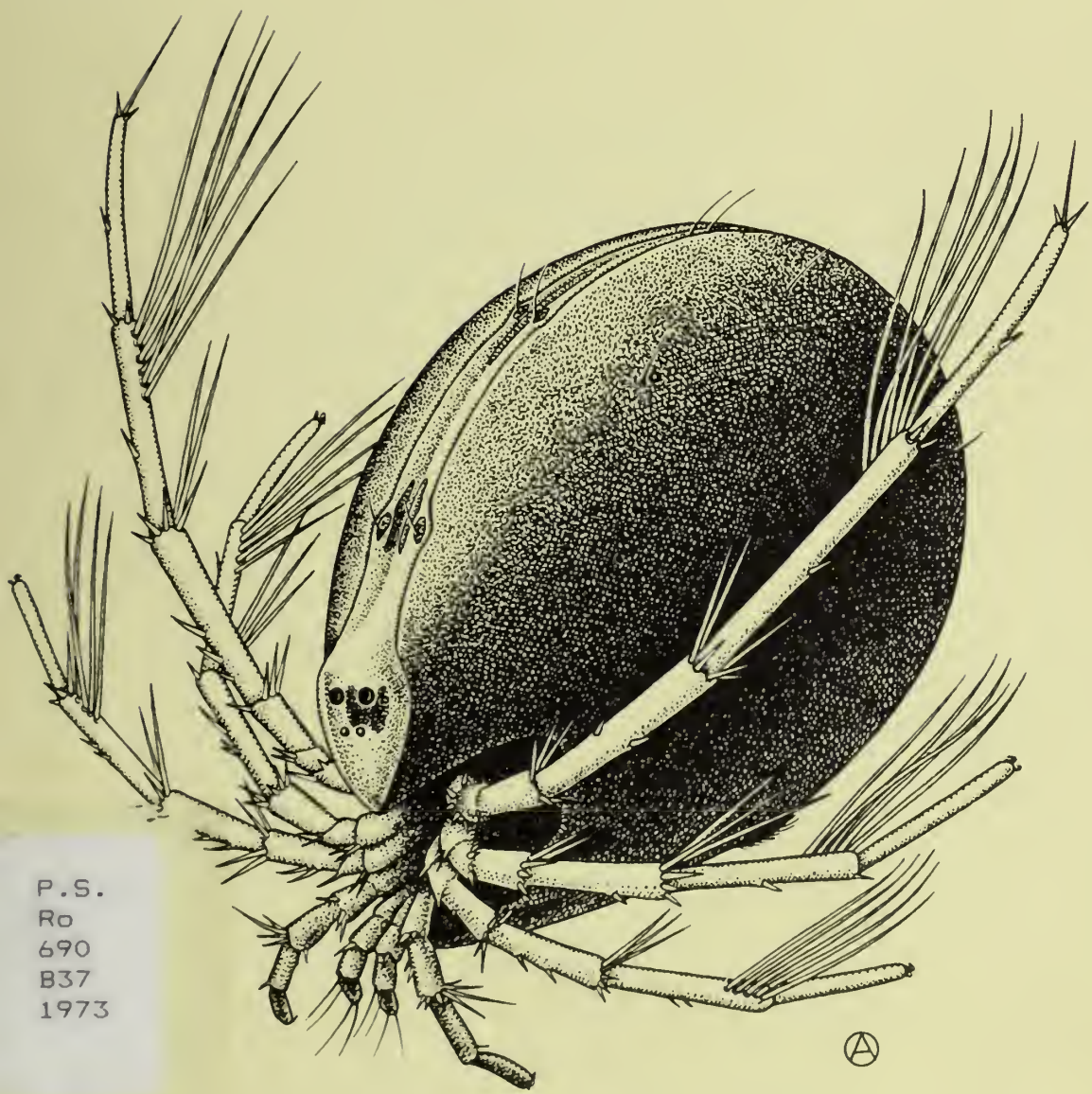
Methods for the Collection, Preservation, and Study of Water Mites (Acari: Parasitengona)

David Barr



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DAVID BARR

Methods for the Collection, Preservation, and Study of Water Mites (Acari: Parasitengona)

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Methods for the Collection, Preservation, and Study of Water Mites (Acari: Parasitengona)

Abstract

Methods required for the systematic study of water mites include new techniques that complement or replace some already in existence. Netting, trapping, and separation procedures are described for mites collected from lentic, lotic, moss, water-film, and phreaticolous habitats. The most useful larval specimens are obtained by collecting gravid females and harvesting larvae hatched from eggs deposited in the laboratory.

Fluid storage is preferable for most specimens. A versatile method of examining exoskeletal morphology is to observe temporary slide mounts with light microscopy (bright-field, interference contrast, or phase contrast). Scanning electron microscopy of desiccated mites enhances the study of surface fine-structure but does not replace conventional techniques of illustration (line drawings, photomicrography).

Résumé

Les méthodes requises pour l'étude systématique des mites aquatiques comprennent des techniques nouvelles qui complémentent ou remplacent quelques-unes de celles en existence maintenant. Des procédés de prendre en filet, d'attraper et de séparer sont décrits pour des mites recueillies des habitats lentiques, lotiques, phréatiques, de la mousse, et de la filme d'eau. Les spécimens des larves les plus utiles sont obtenus en receuillant des femelles enceintes et en récoltant des larves éclosés des oeufs deposés au laboratoire.

Conservation en fluide est préférable pour la plupart des spécimens. Une méthode versatile d'examination de la morphologie exosquelettique c'est l'observation par microscopie optique (illumination ordinaire, interférence contrastée, ou phase contrastée) des montures temporaires en lamelle. La microscopie électronique exploratrice des mites desséchées augmente l'étude de la structure fine de la surface, mais elle ne remplace pas les techniques conventionelles d'illustration (dessins en ligne, photomicrographie).

Introduction

The aquatic families of the suborder Parasitengona are among the most studied taxa of the Acari. Nevertheless, methods of collecting, preparing, and studying these creatures are not standardized, and many useful techniques are either unpublished or scattered throughout acarological literature. I therefore summarize methods found to be useful for systematic research with specimens of water mites. The account is directed primarily to those beginning in the field but also to established workers wishing to explore unfamiliar techniques. Because new methods are continually developed while old ones are being modified and improved, only an interim report is possible.

The treatment is not always comparative, for less satisfactory alternatives are not discussed unless well entrenched in the literature. Nor is the survey complete, for important areas of research (e.g., karyology, transmission electron microscopy, chromatography, electrophoresis) with which I have minimal experience are omitted. But procedures fundamental to systematic, evolutionary, ecological, life history, and zoogeographic studies are covered.

Viets (1936) first compiled methods for water mite study in his general treatment of the German species. There was no other comprehensive survey until those of Cook and Mitchell (Cook and Mitchell, 1952; Mitchell and Cook, 1952) appeared. Other sources of information have been limited in scope. Several authors of general works have described techniques of specific interest to water mite workers (Pennak, 1953; Newell, 1959) or for other mites (Grandjean, 1935, 1949; Newell, 1947; Baker and Wharton, 1952; Krantz, 1970) that can be used for water mites, either directly or with modification. Evans *et al.* (1961) published perhaps the most complete description of general acarological techniques available in English.

Collecting

Most collecting procedures described here involve gathering a portion of the habitat (vegetation, gravel, soil, detritus) and separating the mites from it. Although separation is usually performed in the field, material may be returned to the laboratory for this operation when convenient.

In collecting adult mites, it is worthwhile to strive for quantity. Series of 50–100 specimens of a single species per locality are not too extensive. Not only are large numbers valuable in taxonomic research but are mandatory if mites are to be used as well for sectioning, rearing, karyology, or scanning electron microscope (SEM) observation.

LENTIC HABITATS

Many larger species of water mites occur in standing bodies of surface water – ponds, lakes, and ditches. These mites are easy to collect and have received attention from students of the group for more than a century. Cook and Mitchell (1952) described the dip-netting technique that is most useful for collecting in this habitat, a method particularly effective because most of the mites of standing waters swim well. They readily leave netted vegetation and detritus floated in a white enamel tray and are easily picked up with a dropping pipette and placed in the collecting container.

A useful collecting accessory is a polyethylene dropping pipette with a tapered tip. If progressively larger sections of the soft, plastic tip are sliced off, the corresponding intake bore of the pipette is increased. With several of these pipettes, one can efficiently handle mites of widely different sizes.

Periodically the amount of water in the collecting vial is reduced with the pipette (tip held against the bottom to exclude mites) so that additional specimens can be added. The collection is then transported to the laboratory for biological work; or all water may be pipetted off and the preservative added in the field.

Deep-water (> 2 m), benthic mites (e.g., *Huitfeldtia*) are not accessible with standard techniques and are usually collected using a dredge sampler, a slow process yielding small numbers of specimens. Pieczynski (1962, 1965) employed underwater traps for sampling mites of standing-water habitats.

Certain lentic habitats, notably temporary pools and many boggy situations, contain at the same time large amounts of dead vegetation and detritus and numerous species of mites that either do not swim readily or that crawl on the bottom. As Cook and Mitchell (1952) pointed out, crawling species are difficult to separate by the technique of dip net and enamel tray. Generally, too much detritus is taken in the net. Most specimens do not come out but must be sought by turning over every piece of plant material in the tray. Collection, even of swimming species, is hampered by excess debris. The suggestion of picking over vegetation in the pond (Cook and Mitchell, 1952) is valuable, and in some situations a more specialized technique, developed by Ian M. Smith (pers. comm.) to collect species that do not swim readily, may be helpful. His method involves holding an enamel tray submerged 3–15 cm beneath the surface of the water to form a clear, white background. Working it gently through and under bottom detritus, one can see mites swimming or occasionally crawling across the white background and pick them up, one by one, with a pipette.

LOTIC HABITATS

Numerous mite species also inhabit running water. Perhaps the method proved to be most useful in a variety of lotic situations is the technique (Cook and Mitchell, 1952) of washing submerged vegetation (panning). Masses of aquatic or submerged terrestrial vegetation are collected by hand and transferred to a white enamel tray filled with 3–5 cm of clear water. Vegetation is floated in the tray, taking care that not too much is added to a given tray. Within 5–30 minutes, depending on the initial temperature of the stream water, mites leave the vegetation to swim in the open water or crawl across the tray bottom. The method is slow, particularly when the stream temperature is low (< 15°C) and the appearance of mites delayed. Thus, efficiency is increased by running two or more trays simultaneously. The coarse screen mentioned by Cook and Mitchell (1952) is not necessary and is restrictive when collecting specimens continuously as they appear. It is necessary, however, to move the clumps of vegetation occasionally to discover mites on the tray beneath.

With this technique are taken many species occurring in various microhabitats in the stream other than the truly phreaticolous species that characteristically inhabit gravel several inches below the stream bed (see p. 00). The relative numbers of species found in vegetation differ from those at other locations in the stream, but the species diversity of vegetation collections is sufficiently great to justify this method as the first choice in a new locality when time is limited.

The type of vegetation chosen for panning will vary with stream type. Fine-structured plants are more productive than simple, large plants of gross structure; i.e., aquatic mosses (Musci) are usually superior to *Nasturtium* spp. In turn, fine-structured mosses are usually more productive than species with larger leaves. Mites are also found in clumps and mats of algae, particularly those taken from riffle areas of a stream. If truly aquatic vegetation is sparse, submerged terrestrial vegetation will often be productive; e.g., grasses (Graminae) hanging over the bank, roots of grasses and other angiosperms along the bank and occasionally in the centre of the stream, or islets of grass or other terrestrial vegetation growing on a submerged log or other stable location in the watercourse. The technique of panning submerged vegetation will yield at least a few mites from almost any running water.

A useful method to separate crawling or weakly-swimming mites from vegetation in rheocrene springs and seepage areas has been used extensively by David R. Cook (described in litt.). When examined directly in a pan, this material often contains so much mud and suspended organic matter that organisms are difficult to see. Instead, handfuls of vegetation, including moss, matted roots, and leaves, are placed in a pail or large, wide-mouth jar, and only enough water is added to cover the plant material. Pieces of cheesecloth cut into 10 × 20 cm strips are crisscrossed on top of the material so that at least two or three layers are placed everywhere. If the pail is then not disturbed for a few hours, most mites move toward the surface (perhaps in response to decreasing oxygen levels in the vegetation) into the cheesecloth, which can later be removed strip by strip and examined in a tray of clear water. Mites clinging to the cheesecloth are easily seen and collected.

A stream bed with a depth of 5–30 cm of loosely consolidated gravel often yields many mites, most of which live in the top 5–10 cm of gravel or underneath stones on the stream bed. A method developed by the author has proved to be valuable in a variety of gravel-bed stream habitats. A cone-shaped (base:height 1:3) net of 125 μm plankton netting is held by hand in the stream, with the mouth opening upstream. The opening is covered with coarse aluminum window screening (seven wires/cm) to exclude stones and large pieces of debris. The collector stands upstream from the net and, with his boots, vigorously disturbs the substrate to a depth of 15–20 cm. Still holding the net under water, he slowly collects upstream for a distance of 3–4 m. The net is then withdrawn from the water and is held up so that sediment drains to the bottom. Additional water is splashed into the top of the net to wash down the sediment.

All sediment collected is then distributed evenly over the bottom of a white enamel tray containing 3–5 cm of clear water. Material collected,

which is composed of fine gravel, silt, and other detritus, should form a layer no more than about 3 mm deep when spread over the pan. Mites begin to leave the sediment as soon as it settles, climbing over the surface and burrowing through the material. Many species that live in this habitat are small but usually can be seen against the dark substrate because of the dorsal white or pale spot that marks the site of the excretory gland. Species lacking a visible excretory gland are often otherwise conspicuous because of coloration or movement. Many smaller mites (e.g., *Aturus* spp.) swim actively above the substrate and may even reach the surface of the water in the collecting tray. Detection of specimens is nonetheless a problem, and collecting under a heavy overcast or in rain is difficult.

The plankton-net technique is superior to the screening method described by Cook and Mitchell (1952), for many specimens pass through coarse insect screening without clinging to the wire. In a productive gravel-stream habitat, a comparative test of the two methods clearly demonstrated the superiority of the modified plankton net (Barr and C. A. Lanciani, unpubl. data). It offers probably the best way to obtain the large samples (up to several hundred specimens per net sample) required for diversified research needs. Even so, dip-netting should not be neglected in the stream habitat, especially in areas of marginal vegetation and silt deposits in slow water.

Most lotic species are relatively small and can be readily collected with a small, glass, dropping pipette (medicine dropper type). Because many stream mites are adapted to cling to smooth surfaces in a current (often with the aid of secreted mucilaginous substances), they easily become lodged in the pipette and may be difficult to extract. But individuals usually detach when water in the pipette is allowed to rest momentarily, and the mite again floats or swims. Then water in the pipette is rapidly expelled into the collecting vial.

Experience has shown that not all suitable-appearing streams have a rich gravel fauna. As many different collecting techniques should be tried at each locality as time permits. In many streams and rivers and along rocky, wave-swept lakeshores, water mites occur on the underside of flattened rocks lying on the gravel-based substrate. By carefully examining the surface of the rock after removing it from the water, specimens can be detected and picked off, either with fine forceps or a sharpened toothpick as described by Cook and Mitchell (1952). Collecting by this method, however, is tedious. Crowell (1960) described a method of brushing the underside of the rocks into a pan of water and collecting the dislodged mites with a pipette. Specimens can also be obtained by merely placing the stone in a pan of still water and allowing mites to leave of their own accord. Most rheophilic mites begin to move when placed in still water, more actively as the temperature of the water increases and oxygen content drops.

Water mites are usually among the fauna captured by drift nets placed in running waters (e.g., see Clifford, 1972). Drift sampling may represent a means of obtaining data on the composition of a stream mite fauna but is unlikely to reveal diversity as well as several of the methods described above.

WATER-MARGIN HABITATS

The water-margin habitat supports several highly-specialized species (Mitchell, 1960), the most common being *Tyrrellia* spp. These mites, which live in a film of water less than the thickness of their own bodies, can be collected slowly and tediously by scanning a prospective habitat visually from a distance of 20–40 cm. Specimens are removed from the surface one at a time with a pair of fine forceps. Appropriate situations are beds of moss kept moist by spray from falls or rapids, moist banks of streams and lakes (particularly wet bits of wood), stretches of mud-bank, and the stem bases of semi-terrestrial vegetation near the water-line. The surface must glisten with a thin film of water to be suitable for mites. Many water-film species are bright red and easy to see against the background, but *Tyrrellia* spp. are occasionally dark and inconspicuous.

MOSS HABITATS

Clumps of moss (Musci) growing on stones or wood submerged in flowing water may harbour populations of unusual and often primitive genera of mites (e.g., *Trichothyas*, *Panisus*, or *Hydrovolzia*). Often the most productive moss samples grow at the air-water interface along banks or on projecting objects in the centre of the water where they are always abundantly saturated but never submerged. The same productive habitat may occur in wet areas created by springs or in the spray zone of rapids or waterfalls.

Species found in saturated moss are usually sessile and will not leave the vegetation even if the sample is submerged in still, shallow water indefinitely. Two methods can be used to extract them from the sample, one simpler and more straightforward than the other. Although both techniques are equally effective, both are biased for certain taxa.

The first is a simple field expedient. A clump of moss is held in one hand and systematically picked apart, strand by strand, with a pair of iris forceps. Most species found in mosses are bright red or orange, are easily seen near the base of the stalks of moss, and can be removed with little difficulty using forceps. Eggs and imagochrysalids attached to leaves and branches of the moss can be removed only by breaking off a small section of the plant and placing it in a collecting vial. Adding vegetation also gives living mites in the vial additional surface area for climbing, thus reducing the chance of encounter and consequently of damage by predation. With the hand-picking technique, large numbers of adult and immature mites, coloured red, orange, yellow, pink or even brown, are detected. Dark coloured specimens, such as some *Laversia* spp., however, could be missed in this type of scanning.

A second technique is more likely to yield cryptically coloured mites (I. M. Smith, pers. comm.). Moss is rapidly shredded, strand by strand, into a tray of still water. When the entire sample has been immersed, the water is stirred and vigorously swirled around the tray to allow all mites to become detached. Then loose moss is gathered, rinsed slightly as it is removed from the water, and set aside. When the water clears, numerous mites can be seen lying on or crawling through a layer of brown sediment that has settled on the bottom of the tray. Specimens can then be collected rapidly. Although dark-coloured mites will be free and actively crawling,

they still may be difficult to see against the dark background of sediment. The method does not, however, facilitate the collection of wholly sessile life history stages.

Both techniques were tried on moss samples from the same locality, subsequently subjecting discarded moss to examination by the alternate technique. In each instance a number of mites missed by the first method were taken by the second. At present, data are lacking to indicate the relative efficiency of the two techniques. Hand-picking is simpler in the field, but both methods require good lighting for effective scanning.

PHREATICOLOUS HABITATS

The diverse mite fauna of interstitial (subterranean) waters can be collected by the method of Karaman-Chappuis (Motas, 1962). First, a hole deep enough to penetrate the water table is dug in the bank of a stream. The hole fills with water, which is scooped out and passed through plankton netting to concentrate specimens. Water removed from the hole is replaced by flow from ground water percolating through the sand or gravel interstices and carrying with it the fragile, colourless mites of the phreatic habitat. Specimens are best removed from the accumulated debris under a dissecting microscope. Cook published on North American collections (e.g., 1963, 1968) taken in this manner and found poorly consolidated gravel bars along the banks or in the centre of streams to be particularly productive.

HOSTS

Studies of water mite life histories and host-parasite interactions demand considerable effort and ingenuity in collecting parasitized host insects. From the host can be obtained larvae, larval sclerites and, often, associated nymphs. There is, of course, no general collecting technique. The host of the species of interest must be determined and then standard entomological techniques (Oldroyd, 1970) applied to collect that host. Although the incidence of parasitism is occasionally high, especially with the aquatic Hemiptera, it is often less than 10% (e.g., Coleoptera; Lanciani, 1970).

Aquatic hosts are efficiently collected with the dip net and enamel tray described for collecting adult water mites. A useful accessory to remove fast-swimming Coleoptera and Hemiptera from the collecting tray is a small tea strainer. Once isolated in the strainer, the specimen may be knocked into a collecting jar or removed with forceps and preserved. Oldroyd (1970) described an underwater light trap for capturing aquatic Hemiptera (also see Pieczynski, 1962). Aerial hosts may be netted on the wing or at rest by sweeping vegetation close to the banks of the body of water. They may also be taken with black (ultraviolet) light or in various types of emergence cages.

Engorged larvae or nymphochrysalids removed from hosts may be satisfactory for identification but are usually too damaged or deformed to be valuable for taxonomic descriptions.

LARVAE AND EGGS

Larvae are usually collected in the field only incidentally and may be discovered in collections of adults at the time of initial sorting. Larvae can

occur in stream collections taken with a plankton net, probably having been mistaken for adults of some small stream species, and collected with the pipette. Occasionally larvae occur in large numbers on the surface film of standing waters, where they may be collected with any type of cloth net swept across the water with the lower edge just beneath the surface. The net must be everted into alcohol immediately upon removal from the water to kill the larvae and prevent them from swarming out.

When picking through moss samples, larvae will occasionally be noticed climbing over the vegetation. Single larvae may be picked up with the tip of a pair of fine forceps or a fine camel's hair brush dampened in 70% ethanol.

Eggs also are not commonly sought in the field but may be observed in numbers in pond and moss habitats. Egg masses should be taken with a bit of vegetation to the laboratory, isolated in a small container of water, and held until the time of hatching. Field-collected larvae and egg masses are less useful taxonomic material at present, because in the absence of larval descriptions, only circumstantial evidence is available for association with the adult form.

Preservation of Adults and Nymphs

For taxonomic purposes the best preservative for adult and nymphal water mites is modified Koenike's solution (GAW; see Appendix) as recommended by Mitchell and Cook (1952), for specimens preserved in alcohol or formalin are more difficult to clear (see p. 12).

GAW-preserved specimens of most water mites are flexible, yet firm, and are easily cleared in acetic corrosive (André's fluid) or potassium hydroxide solution (Mitchell and Cook, 1952). The resulting preparations are strong and transparent. So robust are GAW-preserved specimens that they are easily handled, and breakage of specimens caused by routine transfer or dissection is rare. Mites with large areas of weak, membranous integument (e.g., large *Eylais* spp., *Limnochares* spp., and *Wandesia* spp.) become fragile in GAW, but at least the sclerites are easily cleared. In practice, adult mites are stored indefinitely in the original preservative until they are cleared for detailed study.

When adult mites are prepared for the study of internal morphology, a histological fixative is recommended. Several standard reagents, for example Brasil's fluid (Mitchell, 1964), are satisfactory, especially for soft-bodied mites. Often, however, in the preparation of serial sections, hard sclerites of the body wall interfere with the cutting process. Adjacent areas of the section are often damaged, or the entire section may be destroyed. One solution involves embedding in a plastic medium (De Giusti and Ezman, 1955; Woodring and Cook, 1962).

To eliminate these disadvantages more simply, adult mites can be preserved with a fixative that hardens soft tissue but does not unduly harden sclerotized structures. Such a fixative (modified Carnoy's), described by De Giusti and Ezman (1955), has proved to be satisfactory in preparing hard-bodied, sclerotized mites for sectioning. The fixative should be pre-

pared freshly each time. After one week in the fixative, specimens can be washed and stored in 70% isopropyl alcohol until ready for embedding.

Larval Mites

REARING

A prime requisite in collecting and preserving larval mites is that larvae be readily associated with adults of the correct species. An unambiguous association makes identification possible and permits further study of the biology of the species. The single most satisfactory method of obtaining larval material for study is to rear eggs laid in the laboratory by female mites.

Females, obtained in the field by any of the methods previously described, are taken to the laboratory as rapidly as possible and isolated in small containers of water. Two-dram shell vials containing about 1 cm depth (i.e., 1 ml) of water proved satisfactory for most species reared in this laboratory. A short (1–2 cm) strip of filter paper placed in the vial seems to stimulate oviposition in some species. Females are then held at room temperature (which in Toronto may rise to 30°C in summer) and observed every 3 days to determine if oviposition has occurred or if water must be added to replace loss through evaporation. Whenever possible, the original water is from a clean lake or stream near the laboratory, but the level is maintained with glass-distilled water.

Some mites collected from temporary ponds shortly after the ice melts or from cold springs and streams may survive better if maintained at 5–15°C. Cooling is occasionally necessary to keep the female alive, although low temperatures also severely retard the rate of embryonic development.

Several systems of organizing females and reared larvae have been tried in this laboratory. The most convenient to date consists of holding isolated females in rearing vials in clear plastic boxes grouped by collection. A shallow box (approximately 55 × 120 mm) that holds 27 two-dram vials can be placed easily under a dissecting microscope and scanned to check each specimen. Scanning should be done every 3 days, watching for either oviposition or death of the mite in the vial. If a mite dies, it is removed and preserved in GAW. If oviposition occurs, the vial is separated with others containing eggs by a cardboard divider. The female is left with the eggs until they hatch or until she dies. If the female dies before the eggs hatch, she should be removed, preserved in GAW, and assigned a rearing number that is also given to the vial of unhatched eggs. This number allows a female and the larvae reared from her eggs to be associated subsequently and also identifies individual rearings within monospecific series from a given habitat.

Although only females have been discussed, an advantage of the system described is that when space and equipment permit, entire collections of mites can be set up without preliminary field sorting. They are scanned as before. Gravid females that oviposit are separated from non-ovipositing mites, and the rearing process is followed to completion. Non-gravid females, males, and nymphs, are simply preserved in GAW at the time of death and stored in a single vial kept for that collection. The only additional processing required for non-reproductive specimens is the time spent scanning them.

Vials in which oviposition has been observed should thereafter be checked more frequently. During embryonic development, partially-developed embryos can be harvested if required.

HARVESTING AND PRESERVATION

Larvae are harvested as soon as possible after hatching. Prasad and Cook (1972, p. 5) discussed techniques to extract eggs from the female prior to oviposition and to assist larvae to leave the egg membranes. Normally, larvae are removed from the rearing vials and preserved, and the female is preserved in GAW, both components of the collection receiving the same permanent rearing number.

When it is suspected that a female being held for oviposition will produce aerial larvae, rearing is probably best carried out in a stoppered vial (aquatic larvae are reared in open vials). Soon after hatching, aerial larvae may be crawling everywhere on the inside of the vial. To facilitate harvesting, 0.5 ml of 70% ethanol is added to the vial, the stopper replaced, and the vial agitated thoroughly. Alcohol reduces the surface tension of the water, allowing the larvae to be wetted. When contents of the vial settle, all larvae will probably be submerged and partially paralyzed by the alcohol, preventing them from passing through the surface film again. From this point aerial and aquatic larvae are treated alike.

Larvae, first drawn from the rearing vial into a Pasteur pipette with as little water as possible, are then pipetted into the depression of a white porcelain concavity plate. Pipettes should be rinsed frequently between transfers to avoid mixing larvae from different females. From the concavity plate, larvae are transferred singly (or two or three at once if possible) to a polyethylene microvial (Fig. 1A) filled to the brim with a solution of 1 part glycerin in 9 parts 70% ethanol, a glycerine-based fluid with a surface tension sufficiently low to allow larvae to be submerged easily. Transfer is effected with a microloop constructed from a bent minuten pin (Fig. 1B). Such a loop has proven useful for most operations involving the transfer of larvae or of dissected adult structures.

Polyethylene microvials employed are sold by some entomological supply houses as "genitalia vials" and are also available from industrial suppliers (e.g., Caplug Division, Protective Closures Co., Inc., Buffalo, N.Y.) as "tubing caps." Transfer of larvae from water to preservative, perhaps the most time-consuming segment of the operation, provides for minimum dilution of the preservative and eliminates any need to concentrate specimens before final storage. Microvials are left unplugged overnight to allow alcohol and water to evaporate. The larvae remain in a small drop of glycerin at the bottom of the microvial and so preserved can be stored indefinitely.

Long-term storage in glycerin apparently has only a minimal effect on larval specimens. Some internal maceration may occur, but further clearing is invariably required before mounting for observation with transmitted light. Legs, setae, and integument remain reasonably supple.

Microvials are ultimately stoppered with a tiny cork stopper (no. 000, 4X grade) to keep out dust and other foreign material. No particular seal is

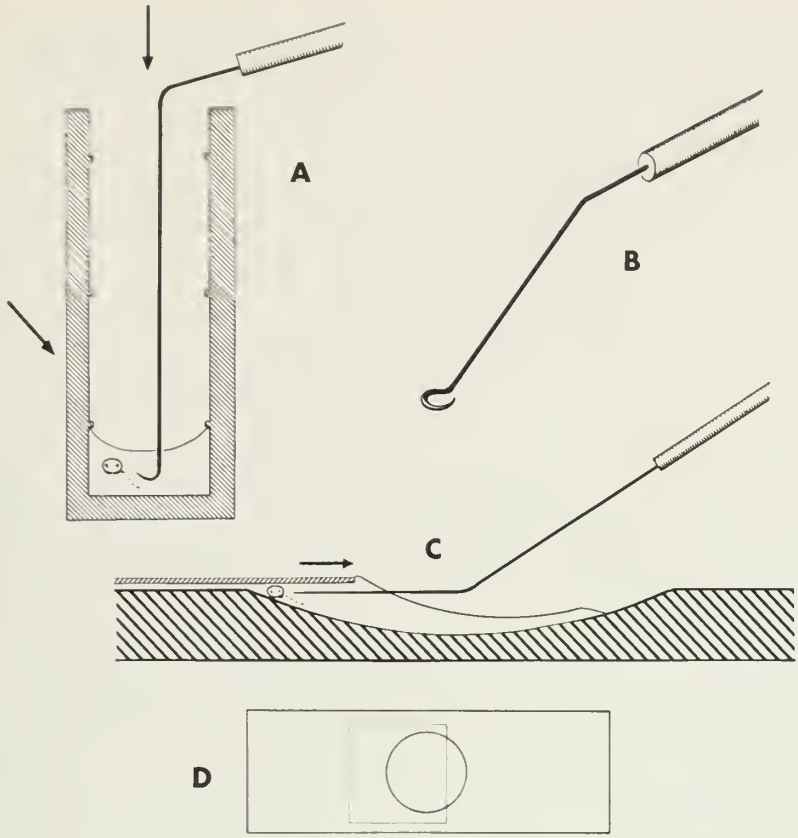

Fig. 1 A. Retrieval of specimens from a polyethylene microvial. Upper arrow indicates direction of observation through dissecting microscope, lower arrow, direction of incident illumination.

B. A wire microloop for transporting minute specimens.

C. Preparation of a glycerin temporary mount on a concavity slide (after Evans *et al.*, 1961, fig. 105). Arrow indicates direction of coverslip motion.

D. Completed temporary mount on a concavity slide (after Evans *et al.*, 1961, fig. 104).

required, as glycerin does not evaporate appreciably. Cork stoppers tend to shed fragments onto the surface of the glycerin; neoprene stoppers supplied with plastic genitalia vials are cleaner but more expensive (approximately three times the cost of microvials).

Microvials are stored dry in a larger glass vial containing the various data labels that accompany the collection. A microvial can also be stored on its side, mounted on an insect pin thrust through the cork, because hydrophobic polyethylene prevents glycerin from creeping along the wall. The glycerin drop remains immobile in the bottom of the vial, holding the specimens there until needed.

The final step in preservation of the associated female and larvae is the transfer of the female from GAW to a drop of glycerin in a polyethylene microvial. The microvial can then be placed dry in the same larger vial containing larvae and labels.

PRACTICAL CONSIDERATIONS

For most rearing operations (sorting field collections, handling adults and larvae) a low-power, stereoscopic dissecting microscope with continuously variable (pancratic or zoom) magnification is recommended. The lower extreme of the magnification range should be as low as 10X, and rarely (including, for the experienced worker, most generic determinations) will magnifications greater than 40X be required.

The entire operation of rearing mite larvae is time-consuming, and to carry out a large-scale program in the field during extended collecting trips is inadvisable; three days' work on the rearing material is required for each day to collect adults. Field rearing, however, may be necessary for rare mites or for species of particular interest. But the most efficient arrangement is to maintain both a field party collecting adults and a support staff rearing and harvesting in the laboratory.

Field collections receive an initial sorting (although they need not) and are mailed to the laboratory (airmail if necessary) in capped vials in a sturdy, protective, mailing container. Mortality is low when mailing time is no more than 2 or 3 days, but larvae from females that were as long as 5 days in transit have been successfully reared. It is essential when packing mites for shipment to provide a high level of dissolved oxygen by filling the vials no more than half full of water (short, wide vials also improve the surface:volume ratio) and by drastically restricting the number of mites per vial. Only 10–15 medium-sized mites (e.g., *Limnesia* spp., *Piona* spp., or *Arrenurus* spp.) travel successfully together. Even smaller numbers (2–5) per vial will be necessary with large species, such as some *Hydrachna* and *Eylais*. For cold-water species, it might be advisable to insulate the shipping container and possibly to provide some means to cool the contents in transit. Most adult mites, however, tolerate high temperatures for some time, and we have successfully mailed and reared cold-water mites at ambient summer temperatures.

If the duration of the collecting trip is no more than a week, collections may be stockpiled in an ice chest (5°C±) and sorted for laboratory rearing on return. Some oviposition may occur in the field, and larvae from these eggs cannot be positively associated with a specific female.

Observation and Final Storage of Skeletal Preparations

EXOSKELETON OF ADULTS AND NYMPHS

Sorting and Clearing

Adult mites preserved in GAW are easily cleared for microscopic examination. Initial sorting by an experienced worker may be carried out under a dissecting microscope without clearing. For critical study, species determination, and separation of similar or closely related genera, a cleared speci-

men mounted on a slide should be examined by transmitted light at 100–400X. Acetic corrosive (André's fluid), described by Mitchell and Cook (1952), is satisfactory for clearing most adult water mites preserved in GAW. The other clearing agent also mentioned by those authors (ibid.), 10% potassium hydroxide (KOH), should be used with the several families of heavily sclerotized mites having characteristic pigmention of the dorsal sclerite (e.g., Torrenticolidae, Aturidae, Axonopsidae, Mideidae, Mideopsidae, Krendowskijidae, and the genus *Koenikea*). Such patterns are obliterated by acetic corrosive but are unaffected by gentle KOH clearing.

The technique of Essig (1948) for clearing alcohol-preserved specimens is satisfactory, but the process is tedious. Possibly the enzymatic maceration described by Newell (1947) could also be valuable in reclaiming adult mites preserved in improper fluids.

Dissection

Specimens can be dissected with only a few, specialized instruments. Most appendages are easily removed by inserting a fine needle (e.g., no. 00 insect pin) at the base; only a small amount of pressure is needed to separate the appendage from its socket. A difficult dissection is the separation of dorsum from venter in species lacking a sclerotized dorsal plate. Large specimens ($\geqslant$ 2 mm in diameter) can be dissected readily into dorsum and venter with a pair of fine, surgical scissors. Scissors cannot be used on mites less than 1 mm in diameter, although other workers have had success with sharpened needles or glass scalpels (pers. comm.). Separation of dorsum and venter is important primarily in large or heavily-sclerotized mites where the presence of one surface seriously impedes the transmission of light through the surface being examined. Usually, in heavily-sclerotized species, a well-marked line separates the dorsum and venter, and the insertion of needles at several points along this membranous zone will separate the two halves of the body completely. In smaller, lightly-sclerotized species, such interference is minimal, and these specimens can be successfully studied in temporary mounts without extensive dissection.

Classical Slide Mounting

The classical method of preparing specimens for observation and permanent storage (Mitchell and Cook, 1952) consists of a double-coverslip slide mount containing a dissected specimen embedded in glycerin jelly, a medium of low refractive index. Although these mounts are easy to examine initially and provide a high degree of permanence for museum collections, they have several disadvantages. After 10–15 years, specimens preserved by the double-coverslip technique tend to accumulate an external deposit consisting of orange-brown droplets that often obscure structural details. A disadvantage common to all permanent mounts is that structures cannot be further manipulated or dissected. A related restriction is that the relative thickness of many such preparations precludes the free use of oil immersion and some "high-dry" objectives. A final and perhaps most compelling disadvantage of the permanent mount is the considerable amount of time required to prepare each specimen.

Fluid Storage

Permanent preservation in glycerin eliminates the difficulties described for permanent, double-coverslip slide mounts and has few serious disadvantages. Adult mites are cleared and then stored in 96% glycerin, either in groups in large vials or singly in polyethylene microvials (see p. 10). To examine the specimen, it is removed from the microvial, placed on a concavity slide in a drop of glycerin, and treated as a temporary mount. The main disadvantages are the handling time required for each observation and the difficulty of maintaining numerous temporary mounts simultaneously during a taxonomic study. The second restriction may be eased by routine and consistent use of black-and-white photography during an investigation. Much sorting and gross inspection can be done with photographs, resorting to the specimens only for critical details.

Storage in fluid allows any of the standard dissections needed to examine discrete organs (*viz.* removal of dorsum, capitulum, palp, leg, or ejaculatory complex). Most parts can be stored together in the same drop of glycerin in a microvial; each can be retrieved individually with the proper tools. Although the ejaculatory complex can be stored with other structures (see p. 16), because of its great transparency and small size it may be easier to recover if stored separately in its own microvial.

To retrieve specimens, the microvial should be uncorked and placed vertically on the stage of a dissecting microscope. With practice, the container can be held in the fingers for this operation or placed in a rack or stand, holding it firmly under a strong source of light (Fig. 1A). Illumination is through the side or from below; translucent polyethylene walls disperse light, illuminating the interior of the vial so that even the smallest parts are visible. Smaller organs are worked up through the glycerin with a straight pin and then taken through the surface by simple adhesion to the side of the pin. Larger structures (legs, capitulum) can be removed with a minuten-pin microloop similar to that described for transferring mite larvae (Fig. 1B). The largest parts (dorsum, venter, whole mite) are also retrieved with the loop or with a minuten pin hooked at the tip. Retrieval of specimens can be facilitated if the upper part of the microvial is sliced off so that total height is about 1 cm. A shorter vial is still satisfactory for permanent storage.

I advocate glycerin (96%) for long-term storage of larvae and adults. The low volatility and high density of this inert fluid make it ideal to protect minute chitinous specimens from chemical and mechanical damage. The polyethylene microvial is also resistant to damage and should further protect the material. Thus, the method seems to satisfy the requirements for indefinite museum storage of important taxonomic materials (types, voucher specimens). Entomologists, of course, have stored genitalia preparations in glycerin for some time with apparently satisfactory results (Beirne, 1955, p. 69).

Temporary Mounts

A specimen stored in fluid can be transferred to a drop of glycerin in a concavity slide and observed as a temporary mount. For examination at low

power or with a high-dry objective of 1–2 mm working distance, no coverslip is required. If the surface of the glycerin is approximately flat, illumination and resolution will be sufficient for routine examination.

A temporary mounting technique allowing critical observation (Grandjean, 1949) is commonly used by acarologists to study terrestrial mites. The specimen is placed on a concavity slide near one edge of the depression. A square coverglass is laid over that edge of the depression so that glycerin is drawn under it by capillary action (Fig. 1C). The coverslip is slid farther across the depression, trapping the specimen beneath it, while a minuten pin inserted beneath the slip keeps the specimen in place near the edge of the depression. Glycerin is added when needed to prevent formation of an air bubble. The preparation is complete when the leading edge of the coverslip is about two-thirds the distance across the depression (Fig. 1D), with the specimen wedged between the coverslip and the sloping bottom near the edge (Fig. 1C). In this position, small structures can be oriented by slight movements of the coverslip. If the coverslip is later fixed in place on the slide with three or four drops of clear nail polish or wax, the preparation can be safely examined with an oil immersion objective.

Semi-Permanent Mounts

For a reference collection of identified material, slide-mounted specimens provide rapid and easy access and require a minimum of experience for examination. A collection of slides can be quickly accumulated by mounting adult mites in Hoyer's Medium (Wards Natural Science Establishment, Inc., Rochester, N.Y.). These slides are not permanent even though coverslips are sealed with an impervious ringing compound such as Murrayite (Fisher Scientific Co. Ltd., Montreal, Que.). But for short term (10–20 years) use, they are satisfactory. Additional coatings of the sealant after 1–5 years probably extend the useful life of the preparation.

A major problem with single-step mounting techniques is that they do not allow for orientation of dissected parts (Mitchell and Cook, 1952). If the mount is prepared in two stages, however, perfect placement of structures is possible. A large drop of Hoyer's medium (the exact size determined by experience) is placed on a slide, and in it are placed the dissected structures of the mite. These structures may be transferred from 70% ethanol, water, or GAW and should have been cleared before mounting. The parts are then arranged as described by Mitchell and Cook (1952), excess mountant is removed by drawing it across the surface of the slide with a needle, and the preparation is set aside under a dust cover to dry. In dry weather, after a day or two at room temperature, the mounts will have hardened and the specimens are covered by a thin film of the medium. Occasionally air bubbles develop under sclerites and within appendages, and the value of such mounts is reduced.

Next, a second drop of Hoyer's medium is placed on the dissected parts (now glued to the slide), and a coverslip is lowered gently onto the preparation at once. Minimal pressure is applied during the final seating of the coverslip because fresh medium rapidly softens the hardened film, and

parts can still be displaced. The slide is then dried (1–2 weeks at room temperature or 3–5 days at 40°C), and the coverslip is ringed with Murrayite. Water-soluble ringing compounds should be avoided, because they do not protect the slide from further desiccation or excessive hydration.

It is important that the amount of hardened medium covering the specimen after the first operation be small; sclerites must appear as structures raised above the surface of the slide, with the medium only covering them. Then, when the final drop of medium is applied, the coverslip can settle down to the surface of the specimen. The thickness of the resulting mount is comparable or superior to those prepared by the double-coverslip technique.

Permanent Mounts

A good method (Newell, 1947) of preparing permanent mounts of dissected mites utilizes Hyrax (Van Waters and Rogers, Inc., Seattle, Washington). This medium is permanent, has a resinous base, does not yellow appreciably with age (I. M. Newell, pers. comm.), and has a favourable refractive index (1.65) for mite exoskeletal parts. Hyrax can be employed in a two-step mounting process as described for Hoyer's. Dissected parts are first dehydrated (70% ethanol, 95% ethanol, beechwood creosote) and transferred to xylene before being placed in a drop of Hyrax on the slide. Hardening time for each stage is longer than with Hoyer's but can be decreased by drying at 40°C.

Hyrax may be replaced in permanent mounts by Canada balsam or other resinous medium. Balsam, however, becomes yellow with age and has an unfavourable refractive index for mite chitin (Mitchell and Cook, 1952); but, with the availability of phase and interference contrast microscopes at present, balsam mounts can be studied easily.

EJACULATORY COMPLEX

One organ of the adult mite is unsuited to preservation as a fixed mount. The male ejaculatory complex (EC), because of its small size and intricate, three-dimensional structure (Barr, 1972), is necessarily stored and studied in fluid. Skeletal preparations of the EC are readily prepared from mites preserved in GAW. Soft-bodied species are punctured in the posterior or dorsal body wall, and the EC is teased free with a hooked minuten pin. With hard-bodied mites one must first separate the dorsum from the venter (or remove the caudum for *Arrenurus* spp.) before the EC skeleton can be extracted.

The detached EC is transferred in a drop of glycerin to a white porcelain, concavity plate (16 holes), and subsequent steps are carried out in the various depressions of this plate. The preparation is stained in Eosin-Y in glycerin-alcohol (about 2 minutes) to make it easier to keep in view during the next, or clearing, step in Nesbitt's fluid (5–15 minutes). Clearing must be monitored closely for, if left overlong, the specimen may become too transparent to detect and be lost. After clearing has removed all soft tissues, the preparation is washed in distilled water, restained (Eosin-Y in glycerin-alcohol), passed through 75% ethanol, 95% ethanol, beechwood creosote, and xylene (2 minutes each), and eventually transferred to VH

viscosity immersion oil (R. P. Cargille Laboratories, Inc., Cedar Grove, N.J.). The specimen is handled each time under the dissecting microscope with a minuten-pin microloop (Fig. 1B) and must be transferred rapidly to the next fluid (especially after alcohol steps) to prevent drying. Once in immersion oil, the EC can be transferred with a straight minuten (in holder) to which it will adhere when brought to the surface. Restaining the specimen aids somewhat in observation but is suggested primarily to make it easy to locate after storage in a drop of oil at the bottom of the microvial. Handling the EC skeleton of minute species is simplified if the entire male mite is processed as described above, and the EC is removed only after the specimen is in immersion oil.

The skeletal preparation is studied in a drop of VH viscosity immersion oil on a shallow, glass depression-slide. The temporary mount may be examined without a coverslip. A small mat of cellulose fibers from a facial tissue submerged in the oil forms a suitable, irregular surface on which to place the specimen. The cellulose fibers support and hold the EC, and the high viscosity of the immersion oil ensures that the preparation remains in any orientation at least for several days, sufficient time for observation, drawing, or photography. For critical examination a temporary mount with coverslip (see p. 14) is used, and VH viscosity immersion oil is substituted for glycerin. The ability to orient the specimen by pressure on the coverslip is particularly valuable for EC mounts.

The skeletal preparation may be stored permanently in a drop of immersion oil at the bottom of a polyethylene microvial (see p. 10). The vial is corked and must be stored upright, as the oil will slowly flow along the side of a horizontal vial. VH viscosity immersion oil is advertised to be nondrying by the manufacturer, and I have noted no evaporation in preparations stored for 5 years.

LARVAE

The small size of larval specimens (100–500 μm in length) creates difficulties of storage and observation. Preservation in 70% ethanol plus glycerin and subsequent storage in a drop of glycerin has been described. Specimens stored in glycerin appear to remain in good condition indefinitely.

Perhaps the most satisfactory method for examining larvae is in fresh Hoyer's mounts (see *Semi-permanent Mounts*, p. 15) prepared at the time of harvesting. As many as six siblings can be mounted under the same coverslip. Larvae are initially opaque, but after drying in an oven at 40°C for 3 days to a week, they become thoroughly cleared. The coverslip must be ringed with Murrayite after drying, however.

I have no evidence that Hoyer's mounts continue to shrink with time, eventually pressing the dorsum and venter of the larva closely together, even in slides 6 years old. If dorso-ventral compression were to occur in poorly sealed mounts, a working knowledge of larval anatomy should prevent confusion. Nor have any of the specimens stored for 6 years become overcleared by continuing action of the medium.

Semi-permanent mounts, however, cannot be manipulated or turned to observe different aspects of the specimen, nor can specimens readily be prepared for viewing with the scanning electron microscope. Specimens in Hoyer's can be freed by cracking the coverslip and submerging the entire slide in a small petri dish of water at 40°C for an hour (longer if necessary). The coverslip can then be floated away and the larvae removed.

Larvae preserved in glycerin can be mounted directly in Hoyer's medium with results almost equal to those for a living specimen. Specimens, first cleared in lactic acid (or acetic corrosive) and preserved in glycerin, can also be examined as temporary mounts and rotated, turned, and manipulated to view structures from different angles. Dissection by teasing, or by crushing beneath the coverslip as suggested by Prasad and Cook (1972, p. 6), can aid in the observation of individual structures.

Dried larvae may be the only material available because of accidents in processing, because of drying specimens for the scanning electron microscope, or because larvae were obtained from dried insects in museum or other collections. These specimens can be reclaimed for transmitted light microscopy by softening in lactic acid at 40°C for 24 hours. Larvae can, in fact, be preserved indefinitely in lactic acid, although I prefer to keep all material in glycerin to avoid confusion concerning the preservative used for a given specimen.

Larvae killed by gentle heating and left to macerate in water for several days expand slightly (Prasad and Cook, 1972, p. 6), with the result that setal patterns may be seen more clearly. This procedure is probably not necessary for routine preparations after basic morphology is known.

OPTICAL SYSTEMS

A dissecting microscope and reflected-light illumination suffice to identify most genera of adult water mites and species of the genus *Arrenurus*. Study of most adult mites for detailed morphology requires preparation of the cleared exoskeleton in a temporary or permanent slide mount and observation at magnifications of 100–1000X by bright-field microscopy. In an embedding medium of low (< 1.5) refractive index, most adult structures are too heavily sclerotized for observation with phase or interference contrast systems.

The male EC skeleton, however, requires the highest levels of magnification, and observation is enhanced by increased contrast. Phase contrast microscopy cannot be used successfully on this material as the excessive multiplication of interference fringes obscures detail. But interference contrast (Normarski phase) microscopy improves the image. The thickness and moderate sclerotization of the specimens do not affect resolution, and the detailed structure of membranes, lightly-sclerotized surfaces, and apical setae are more easily seen than with phase or bright-field. Internal structure is also somewhat clearer.

Cleared larvae are transparent and nearly invisible with ordinary bright-field illumination. Phase contrast is generally satisfactory except in heavily-sclerotized areas of the gnathosoma and in lightly-sclerotized structures or

membranous areas. The technique is effective for determining size, shape, and location of setae and of major body sclerites. Interference contrast is equally as effective for setal size, shape, and position and for major sclerites. In addition, somewhat greater detail is visible in heavily-sclerotized areas, in areas where the setae are dense, as in the palpal tarsus, and in lightly-sclerotized structures.

Studies of Internal Anatomy

Apparently techniques for investigating the internal morphology of larvae have not yet been tested. Ultra-thin sections cut from resin-embedded specimens with an ultra-microtome would probably produce informative results.

Adult specimens for gross dissection can be prepared by cellulose nitrate infiltration and then affixed to a paraffin surface (Mitchell, 1964). Qualitative information on shape, size, and location of muscle bundles of the ejaculatory complex can be obtained by examination of the organ, removed before clearing from mites preserved in GAW. Muscle bundles are semi-transparent and only loosely attached to the chitinous framework, so superficial layers can be removed to examine deeper muscle groups.

Serial sections of whole mites require specimens infiltrated with cellulose nitrate and embedded in Paraplast (Mitchell, 1964). Sections are cut at 5–10 μm in longitudinal (sagittal), transverse, and frontal planes. Ribbons are affixed to slides with Mayer's albumin, stained with Mallory's triple stain (Gray, 1964, pp. 111–112), and mounted in Canada balsam.

Mallory's stain tends to fade with time, although less so in acid balsam mounts (Gray, 1964), but for initial morphological studies of arthropods it is invaluable because of the colour distinction given to different tissues. Nuclei stain red, connective tissues blue, glandular tissues blue or purple, muscles red, gonadal tissues red or magenta, and secretions are often blue. The components of the exoskeleton are particularly well differentiated; unsclerotized chitin stains bright blue (hypostracum or endocuticle), lightly sclerotized exoskeleton stains red (ectostracum or exocuticle), and heavily sclerotized areas remain yellow (i.e., unstained). Once various organs and tissues have been identified subsequent preparations should be stained with the permanent dye, iron hematoxylin.

Scanning Electron Microscopy

APPLICATIONS

The scanning electron microscope (SEM) has proven useful for the study of external features of water mites. For adults it has provided micrographs of the entire ventral surface showing sclerites and setae. SEM micrographs yield new information on setae and on structure of small or difficult-to-observe surface features of the body such as acetabula (genital suckers), eupathidia of the palpal tarsus, various glandularia, pores, tip of the capitulum, and eye capsules. Particularly for larvae, scanning electron microscopy is invaluable. Much of the larval observation done with a light microscope is at the limits of magnification and resolution of conventional optical sys-

tems; additional clarification of palpal setation, setal types, sense organs, the anal plate, and other exoskeletal structures is possible only with the SEM.

DESICCATION

To make a satisfactory specimen, the mite must be dried, fixed to an aluminum specimen stub, and vacuum coated with a thin layer of metal or other conductor (e.g., carbon) so that every point on the surface is grounded. With many adult mites simple air drying suffices. Specimens are removed from the preservative (glycerin, GAW, or a histological fixative) and transferred through several (2–6, as needed) changes of 70% ethanol to wash out glycerin or other non-volatile reagents. Fragile specimens may be transferred to absolute ethanol and then to xylene to render exoskeletal structures more rigid and to reduce collapse upon desiccation. After removal from xylene, air drying is rapid. Weakly-sclerotized species of certain genera (e.g., *Atractides*, *Eylais*, *Unionicola*, and *Hygrobates*) and all nymphs benefit by freeze-drying. Aerial larvae are fragile and can be desiccated satisfactorily only by freeze-drying. But aquatic larvae are more robust and can be treated much the same as adults; air-drying directly from xylene is advisable for additional rigidity. Although the air-drying technique for aquatic larvae is rapid, it usually obscures integumental areas, which collapse inward, leaving the heavily-sclerotized plates outermost.

For freeze-drying, specimens should be washed thoroughly in 70% ethanol (at 40°C, six changes in 24 hours) to be certain that all glycerin is removed. The material is then frozen in a gentle, two-stage method. Liquid freon is prepared by spraying the gas from a pressurized tin into a small metal vial floating on the surface of liquid nitrogen. The freon freezes at the bottom of the vial, and a quarter-inch deposit of frozen freon can be built up quickly. The container is then removed and placed on the surface of a laboratory bench at room temperature. Within a few minutes the freon melts but will not evaporate in less than 20–30 minutes. Mite specimens are isolated in small cups of aluminum foil. Excess alcohol is drawn off so that specimens are covered by only a thin film. The aluminum cup is then floated on the surface of the liquid freon, which freezes it almost instantaneously, and after a minute or two the cup is transferred to the surface of liquid nitrogen. Frozen specimens may then be stored in liquid nitrogen or in a freezer, or they may be transferred directly to the pre-cooled stage of the freeze-dryer. After freeze-drying, no particular precautions need be taken with specimens, and they can be stored in the laboratory under atmospheric conditions.

MOUNTING AND COATING

Affixing dried specimens to stubs has been one of the most difficult steps in the preparation of SEM material. Any gluing agent that dries too rapidly is useless because the light, small specimens cannot be transferred to the drop of glue before a thin, dried film forms over the surface of the drop, preventing the specimen from being pushed into it. Consequently, the otherwise favourable silver dag (a dense suspension of silver particles in a quick-drying carrier fluid) cannot be used as a gluing agent for mites. The best

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alternative has been epoxy resin cement applied to the stub in small quantities, each spot no more than 10% of the total volume of the body of the animal to be affixed. Larger quantities tend to migrate up the sides of the specimen, coating it and obscuring most surface detail. Adult mites can be transferred to the dab of cement with extreme care by a pair of fine watchmaker's forceps. But dried larvae are transferred more easily because they tend to adhere to the tip of a dry minuten pin by electrostatic attraction. Once on the liquid resin, the specimens should be slightly pushed into it to ensure firm adhesion.

The hardened resin is totally non-conducting, and so grounding of the surface of the specimen must be thorough. With larger specimens, grounding can be accomplished by painting silver dag (Acheson Colloids Ltd., Brantford, Ont.) around the margin of the specimen to ensure a conductive path from the body surface to the aluminum of the stub. Dag, however, cannot be used for larvae; even the slow-drying variety forms a surface skin too rapidly to allow a drop to be touched to the edge of the larva and drawn out across the stub.

A second method of ensuring good grounding is to vacuum-coat the specimen with a particularly heavy metallic layer (e.g., 400–500 Å) and, if necessary, to coat a second time before observation. Excessive coating does not obscure detail on most structures of interest in water mite morphology. For all mites observed here, pure gold has been the most satisfactory coating agent.

At high magnifications, debris and dirt particles on specimens, particularly those that have been freeze-dried, occasionally impede observation and certainly decrease the quality of micrographs. Specimens can be cleaned and most particulate matter removed by sonication as described by Shear and Levi (1970).

LIMITATIONS

Scanning electron microscopy is not a completely satisfactory method for the study of the external morphology of water mites. Many well-marked sclerites that are easily seen by transmitted light are difficult to observe with scanning electron microscopy because they are partially sunken beneath the surface of the integument. This is also true, of course, for apodemes, ridges, and any other internal structure. Legs and other long, thin appendages projecting from the body are difficult to observe because they cannot be thoroughly grounded; they often charge in the presence of the electron beam and glow too brightly. When using the scanning electron microscope to prepare taxonomic illustrations, it is difficult to orient specimens so that legs and other appendages are well displayed. Moreover, in micrographs of the entire ventral surface, the smaller coxal setae are difficult to distinguish.

Illustrations

LINE DRAWINGS

Black-and-white line drawings are still the most versatile illustration for all phases of water mite study, especially for taxonomic description. Methods

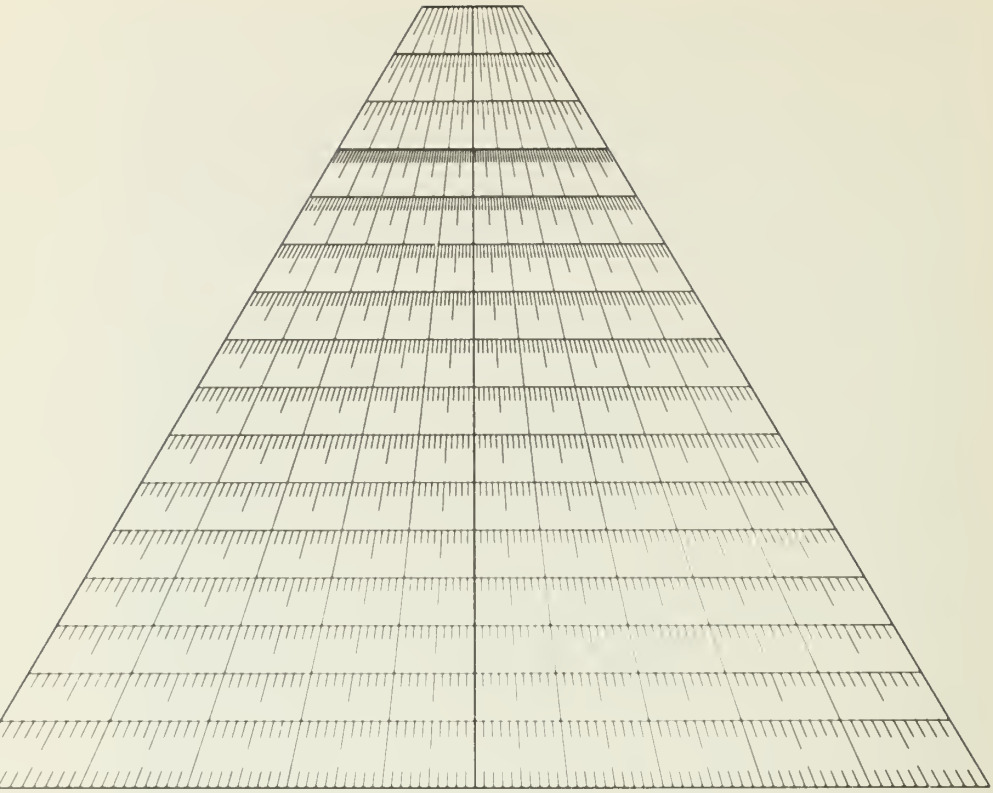

Fig. 2 Enlarged reproduction of ocular micrometer grid used to read setal position directly as a proportion of segment length.

of producing line drawings from microscopic preparations are numerous (projection, camera lucida, drawing tube, squared ocular reticle, tracing a photomicrograph, freehand), and the choice is largely a matter of personal preference.

In preparing drawings of legs, a useful accessory is an ocular reticle of the design illustrated in Fig. 2. This device is used to estimate the position of setae along each segment of the leg to name the position of each seta (Newell and Ryckman, 1966) and to place it accurately on a diagram. With practice, the specimen can be manipulated on the microscope stage until the segment under examination coincides with a horizontal scale line approximately the same length. Each horizontal line is divided into 10 major divisions and as many as 100 minor divisions. Thus, the position of any seta can be read directly as a proportion of the total length of the segment.

In modern acarological literature, legs and leg setae are usually illustrated by detailed, semi-realistic line drawings showing length, structure, and position of all setae. These illustrations are time-consuming to prepare and delay the publication of taxonomic description. A faster method, retaining all information on position and setal type and part of the information on setal

size, was first used for water mite larvae by Barr (1968). Each larval leg is represented by a standardized diagram marked with symbols to indicate the position of the setal bases. Individual setae that are diagnostically important because of size or structure are illustrated separately. Although the value of the setal diagram is only auxiliary in diagnosis, it facilitates interspecific comparison of individual setal positions and of overall setal patterns as noted by Evans (1963) for Mesostigmata. Such diagrams also provide suitable data for quantitative analysis. Because distortion is involved in making each segment fit a standard shape and size, setal diagrams should always be accompanied by an outline drawing of the leg segments marked to indicate the true position of setal bases.

PHOTOMICROGRAPHY

Photomicrography has been important as a method of illustrating water mites. Many of Lundblad's publications (e.g., 1941, 1962) contain photomicrographs of entire ventral and dorsal views as well as isolated views of palp, genital area, and specific leg segments. His photographs were usually supplemented by detailed line drawings of structures of particular interest. Certain water mite structures, e.g., palps, flat dorsal plates, pigmentation pattern in dorsal plates, and often the external genitalia, are more rapidly and better illustrated by photomicrography than by line drawings. By photographing the specimen at low magnification with increased enlargement of the negative, one can take advantage of a greater depth of field than is available at high magnification. A good photograph is a more objective record of shape and relative sizes than many taxonomic drawings.

In studying a specimen or series of specimens, a record set of photomicrographs is valuable. Photographs can be sorted and examined more easily and efficiently than slides, especially temporary mounts, which need only be re-examined in the final stages of the investigation rather than throughout. Photomicrographs can also serve as the basis for line illustrations and for certain quantitative analyses. Moreover, photographs can be used to illustrate variation of shape in a particular structure more expediently than a series of line drawings. Possibly improved scanning electron micrographs will eventually eliminate some of the disadvantages (shallow depth of field, low contrast) of standard photomicrographs.

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Appendix: Formulae for Reagents

1. Acetic Corrosive (André's Fluid) (Mitchell and Cook, 1952)

acetic acid (glacial)	50 ml
chloral hydrate	50 g
distilled water	50 ml

2. Brasil's Fluid (calculated from Baker, 1958)

80% ethanol	150 ml
picric acid	1 g
formalin (37%)	70 ml
acetic acid (glacial)	15 ml

3. Eosin-Y in Glycerin-Alcohol (Barr)

saturated solution of	
Eosin-Y in 95% ethanol	10 ml
glycerin (96%)	10 ml

4. GAW (modified Koenike's fluid)

acetic acid (glacial)	100 ml
glycerin (96%)	500 ml
distilled water	400 ml

5. Modified Carnoy's Fluid (De Giusti and Ezman, 1955)

isopropyl alcohol (abs.)	60 ml
chloroform	30 ml
formic acid (90%)	10 ml

6. Nesbitt's fluid (Evans *et al.*, 1961)

chloral hydrate	40 g
distilled water	25 ml
hydrochloric acid (1N)	2.5 ml

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